frozen bone. Additionally, MSCs were able to adhere to the washed bone, increasing total metabolic activity throughout the time-course, whilst the scaffold itself induced osteogenic differentiation, independent of osteogenic media stimulation. This biological scaffold material therefore has potential for application in bone tissue engineering strategies.

*Disclosure:* The authors have no conflict of interests.

*Reference*

1. Smith CA et al: Biomechanical properties of an acellular bone allograft produced using a novel sterilization process. ORS 59th Annual Meeting (2013) Texas, USA.

## PP540
## Understanding cellular behaviour in early and late stage of MSD

M Ondrésik[1,2], CCorreia[1,2,3], RA Sousa [1,2,3], JM Oliveira[1,2] and RL Reis[1,2]

*[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Taipas, Guimarães, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]Stemmatters, Biotecnologia e Medicina Regenerativa SA, Guimarães, Portugal.*

*Introduction:* Osteoarthritis (OA) is the most common joint disease worldwide with only limited treatment options[1]. It is believed that the thorough analyses of the disease on cellular and molecular base can lead us to the discovery of new targets, and to the development of new therapies. Thus, we have been working on the development of a 3D model of human OA knee cartilage. By this *in vitro* model, it is expected to gain a better understanding on the disease process. The two main aspects of our research are: (1) how the mechanical loading and (2) how the presence of pro-inflammatory cytokines, e.g. TNF-α and IL1-β affects the degenerative processes[2,3]. In the present study, we demonstrated the effect of the different cytokines on monolayer and tissue culture.

*Materials and Methods:* All human cartilage samples were obtained from the local hospital. The cartilage pieces were harvested from the lateral-central regions of both the femurs. The biopsy specimens as well as the cell monolayers were cultured in tissue culture polystyrene (TCPS) multi-well plates, immersed in Dulbecco's modified Eagle's medium (DMEM) (Sigma – Aldrich, USA) with phenol red, supplemented with 10% foetal bovine serum (FBS) (Biochrone, Berlin, Germany) 1% antibiotic-antimycotic solution (Invitrogen, USA) and cytokines TNF-α, and IL1-β (R&D systems – Minneapolis, USA) and incubated for 6 days at 37°C, 5% $CO_2$, within static conditions (ATM 0.1MPA). The cellular viability was assessed by Live/Dead® Viability Assay (C3099, P1304MP, Invitrogen). Cell metabolic activity was evaluated by MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-rboxymethoxyphenyl)-2 (4-sulfofenyl)-2H-tetrazolium) assay (G3581, CellTiter 96® AQueous Assay, Madison, WI, USA). GAG content of the cartilage biopsy specimens was assessed by Safranin O staining.

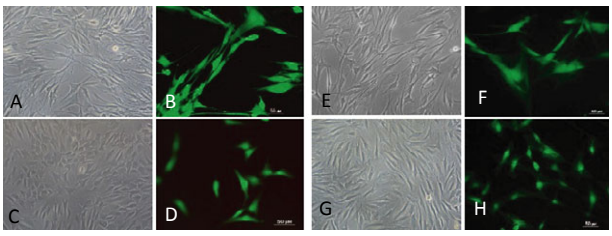


Figure 1. Cell viability - Live/Dead assay. A – B, healthy chondrocytes, 25ng/ml IL-1ß, 100 ng/ml TNF-α; E – F: OA chondrocytes 25ng/ml IL-1ß, 100 ng/ml TNF-α; C – D: control, healthy chondrocytes, G – F: control, OA chondrocytes.

*Results:* The cell morphology of the chondrocytes was altered as a consequence of cytokine treatment. Instead of the roundish phenotype elongated fibroblastic cells were observed after 6 days of incubation with cytokines (Fig.1). Cell viability showed lower rates when cytokine was added in the culture media as it is shown by the Live/Dead assays, however the MTS assay indicated an increasing cell metabolic activity in the presence of cytokines. The tissue culture showed accelerated degradation processes and increased GAG loss when the media was supplemented with cytokines as it was shown by Safranin O and Fast green staining on the histological images.

*Conclusion:* The performed studies showed that the cytokines lead the chondrocytes towards the osteoarthritic phenotype and accelerate the degradation processes in the cartilage tissue as it was expected, therefore ideal agents for the development of the envisioned 3D OA cartilage model. However, further studies are needed to evaluate the effects of cytokines on molecular and gene level.

*Acknowledgement:* This study was performed by the foundation of FP7 Marie Curie Initial Training Network "MultiScaleHuman": Multi-scale Biological Modalities for Physiological Human Articulation (2011-2015), contract MRTN-CT-2011-289897.

*References*

1. M.B. Goldring, S.R. Goldring, Osteoarthritis, *Journal of Cellular Physiology*, **231**, 626–34, (2007).
2. C. Correia, A.L. Pereira, A.R.C. Duarte, A.M. Frias, A.J. Pedro, J.T. Oliveira, R.A. Sousa, R.L. Reis, Dynamic Culturing of Cartilage Tissue: The Significance of Hydrostatic Pressure, *Tissue Engineering, Part A,* **18**, (19–20):1979–91, (2012).
3. M. Kapoor, J.M. Pelletier, D. Lajeunesse, J.P. Pelletier, H. Fahmi, Role of proinflammatory cytokines in the pathophysiology of osteoarthritis, *Nature Reviews, Rheumatology,* **7**, 33–42 (2011).

## PP541
## 3D cellularity within the human knee meniscus

I Fatih Cengiz[1,2], H Pereira[1,2,3,4], J Espregueira-Mendes[1,2,3,4], JM Pego[2], JM Oliveira[1,2] and RL Reis[1,2]

*[1]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Guimarães, Portugal; [2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]Clínica Espregueira-Mendes F.C. Porto Stadium – FIFA Medical Centre of Excellence, Porto, Portugal; [4]Orthopedic Department Centro Hospitalar Póvoa de Varzim – Vila do Conde, Portugal*

*Introduction:* The knee menisci are fibro-cartilaginous tissue located between tibial plateau and femoral condyles in each knee joint, and have important roles in biomechanics of the knee joint including load bearing and shock absorption, secondary stabiliser of the joint and joint lubrication and nutrient distribution [1]. In clinics, complete healing of meniscus still remains as a challenge. Several tissue engineering and other regenerative medicine strategies have been attempting to repair or regenerate the meniscus tissue [2]. Cellularity is one of the important characteristics that should be considered in tissue engineering and regenerative medicine strategies. The aim of this study is to investigate the 3D cellularity of human meniscus.

*Materials and Methods:* Six lateral meniscus tissues obtained from human donors were prepared into sequential 30 μm-thick histological slices and stained with Giemsa. Cells were counted in an in-depth fashion as either fibrochondrocytes or as fibroblast-like cells respectively based on their roundish or elongated morphology (Fig. 1) in a total of 432 regions using Olympus BX51 Microscope and Stereo Investigator

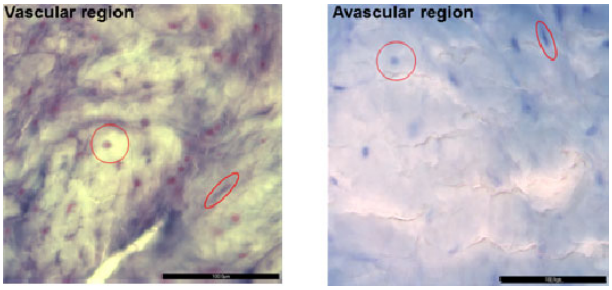


Figure. 1. Histological images of vascular and avascular regions showing different morphologies of the meniscal cells. Scale bars indicate 100 μm.

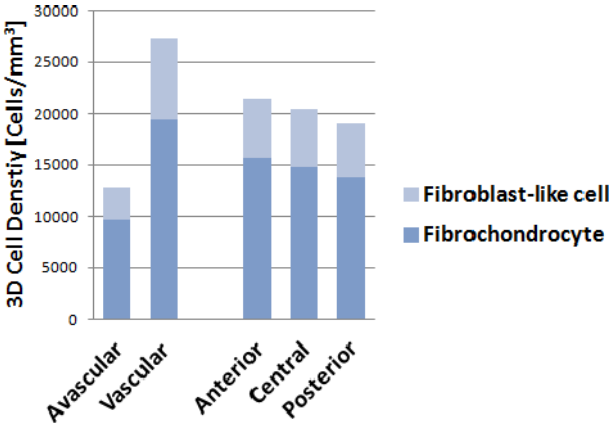


Figure. 2. Mean 3D cellularity of vascular and avascular regions, and across the anterior, central and posterior segments.

software from MBF Bioscience. 3D cell densities were obtained by calculating the number of the cells found in unit volume in the vascular and avascular parts of the anterior, central and posterior segments of the meniscus.

*Results:* Cellularity varies within the human meniscus (Fig. 2), specifically between avascular and vascular regions of the meniscus by having the mean values of respectively 12820 cells/$mm^3$ and 27199 cells/$mm^3$. In average, the abundance of fibrochondrocytes (14,705 cells/$mm^3$) is more than two-and-a-half times as that of fibroblast-like cells (5,539 cells/$mm^3$).

*Discussion and Conclusion:* The clinical phenomena of very poor healing of avascular region and relatively higher healing ability of vascular region could be explained by high cellularity difference in these regions. This work reveals the knowledge of 3D cellularity of human meniscus and provides information to be used in the development of advanced tissue engineering strategies for meniscus regeneration.

*Acknowledgments:* The authors thank the financial support of the MultiScaleHuman project (Contract number: MRTN-CT-2011-289897) in the Marie Curie Actions—Initial Training Networks.

*Disclosures:* Authors have nothing to disclose.

*References*

1. McDermott ID, Masouros SD, Amis AA (2008) Biomechanics of the menisci of the knee. Current Orthopaedics 22 (3):193–201.

2. Pereira H, Frias AM, Oliveira JM, Espregueira-Mendes J, Reis RL (2011) Tissue engineering and regenerative medicine strategies in meniscus lesions. Arthroscopy: The Journal of Arthroscopic & Related Surgery 27 (12):1706–1719.